A Pathogenesis Study of Foot-and-Mouth Disease in Cattle, Using *in situ* Hybridization

Corrie C. Brown, Richard F. Meyer, Harvey J. Olander, Carol House and Charles A. Mebus

ABSTRACT

Eight calves were exposed in an aerosol chamber to nebulized foot-and-mouth disease virus. Two control animals were exposed in a similar manner to cell culture media only. Animals were euthanized at intervals and various tissues examined by *in situ* hybridization using a biotinylated RNA probe corresponding to a portion of the viral gene coding for the polymerase enzyme. By this technique large amounts of viral nucleic acid were found in coronary band, interdigital cleft and tongue as early as six hours postexposure, indicating a very rapid delivery from the portal of entry to the predilection sites for lesion development. This occurred well before the onset of viremia which by virus isolation was not detectable until 30 hours postexposure. The *in situ* hybridization signal in these tissues decreased in intensity and extent with time to focally positive areas, occasionally surrounding a vesicle. Other epidermal sites not normally thought of as sites for foot-and-mouth lesion development, such as carpus and eyelid, also had some viral nucleic acid detectable at various time intervals. In the lung by *in situ* hybridization, alveolar septa had viral nucleic acid early in infection (6–18 h postexposure) while later (36–96 h postexposure), the *in situ* hybridization signal was prominent in alveolar macrophages.

RÉSUMÉ

Huit veaux ont été exposés par nébulisation au virus de la fièvre aphteuse dans une chambre à aérosol et deux animaux témoins ont été exposés au milieu de culture cellulaire. Les animaux ont été euthanasiés à différents intervalles après l'exposition et des prélèvements de différents tissus ont été faits. Les échantillons receuillis ont été examinés par la technique d'hybridation *in situ* à l'aide d'une sonde à ARN biotinylée correspondant au gène de l'enzyme polymérase. Cette technique a permis de déceler de grandes quantités d'acides nucléiques viraux au niveau de la bande coronaire, de l'espace interdigitée et de la langue dès six heures après l'exposition, démontrant ainsi une dissémination rapide du virus vers les tissus de prédilection où se développent les lésions. La détection d'acides nucléiques a précédé l'apparition de la virémie qui a été décelée 30 heures après l'exposition au virus. L'intensité et l'amplitude du signal d'hybridation *in situ* ont diminué avec le temps, et se sont localisées dans certains foyers positifs entourant parfois une vésicule. Des quantités détectables d'acides nucléiques ont été observées à différents intervalles de temps dans certains sites épidermiques normalement supposés indemnes de lésions de fièvre aphteuse, tels que le carpe et les paupières. Au niveau du poumon, la détection d'acides nucléiques tôt après l'infection (6–18 heures) fut limitée au tissu interalvéolaire, tandis que plus tard (36–96 heures) le signal positif fut observé surtout au niveau des macrophages alvéolaires.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral infection of wild and domestic cloven-hoofed animals. Classified as a member of the *Aphthovirus* genus in the family *Picornaviridae*, FMD virus causes a transient yet drastic decrease in production due to the formation of painful blisters in epithelial sites, primarily mouth and feet. Because of its extremely contagious nature, FMD is exceedingly difficult to control and countries which are free of the disease maintain rigid quarantine and import restrictions to prevent its introduction.

The pathogenesis of the disease has been studied by numerous investigators through the use of virus isolation and fluorescent antibody techniques (1–5). Recent technological advances, specifically, the development of *in situ* nucleic acid hybridization for detection of microbial genomes within tissues, have made it possible to trace the path of organisms through the body with greater sensitivity. A biotinylated RNA probe was developed and used successfully for the detection of FMD virus in cell culture (6) and subsequently for the detection of FMD virus nucleic acid in tissues of guinea pigs and pigs infected intradermally with FMD virus (7,8). After intradermal inoculation, FMD nucleic acid was found widely distributed in a variety of epidermal sites, but lesions were largely restricted to areas subjected to mechanical trauma. In addition, both species of animals had considerable amounts of FMD viral nucleic acid in lung, predominantly in alveolar macrophages, 24 to 48 h after intradermal inoculation. These findings had to be interpreted in light of the intradermal route of inoculation, which is not considered to be a natural means of infection for FMD. Inhalation of viral particles is thought to be the most usual method of entry. The aim of the present study was to use the highly sensitive technique of *in situ* hybridization (ISH) to

Foreign Animal Disease Diagnostic Laboratory, National Veterinary Services Laboratories, APHIS-USDA, P.O. Box 848, Greenport, New York 11944 (Brown, Meyer, House, Mebus) and Department of Veterinary Pathology, University of California, Davis, California 95616 (Olander).

Submitted January 27, 1992.

observe the spread of the virus to various tissues subsequent to an aerosol exposure to FMD virus.

MATERIALS AND METHODS

ANIMALS

Ten six-to-nine month old Hereford calves were housed in individual animal rooms and given water and pelleted feed *ad libitum*. Eight animals were exposed to an aerosol of FMD virus in an exposure chamber placed about the heads. The chamber was a one m^3 frame made of copper tubing and covered by heavy gauge plastic sheeting, with a large opening at one end which could be closed around an animal's neck. A small opening on the side accommodated an air supply hose connected to a deVilbiss nebulizer clamped onto the inside of the copper frame, approximately 60 cm from the animal's muzzle and at about the same height. The plastic sheeting remained secured around the animal's neck for a total of 15 min. A compressor supplied air at the rate of 15 psi through the nebulizer so that 2 mL of virus suspension (5.8×10^5 plaque forming units of FMDV Asia 1) were nebulized over the first 10 min. The aerosol chamber became foggy after about 4 min. Animals were monitored clinically, with blood and oesophageal-pharyngeal fluids collected once or twice daily for virus isolation. Animals 1 through 8 were euthanized with Fatal-Plus (Vor-tech Pharmaceuticals, Dearborn, Michigan) at 6, 12, 18, 24, 36, 48, 72 and 96 h postexposure (hpe), respectively. In addition, two control animals (#9 and #10) were exposed in a similar manner to sterile cell culture media and euthanized at 12 and 24 hpe. Numerous tissues (tongue, fore and hind interdigital clefts, fore and hind coronary bands, flank, shoulder, carpus, tail fold, eyelid, lung, teat, heart, rumen, pancreas, tonsil, spleen, hard palate, turbinates, and mediastinal, retropharyngeal, and tracheobronchial lymph nodes) were collected in 4% paraformaldehyde for histopathological examination and in OCT embedding medium (Miles Corporation, Elkhart, Indiana) over liquid nitrogen for *in situ* hybridization. These latter tissues were stored at −70°C prior to sectioning.

HISTOPATHOLOGY

All tissues were examined by light microscopy after embedding in paraffin, sectioning at 4 μm and staining with hematoxylin and eosin.

PREPARATION OF PROBE AND *IN SITU* HYBRIDIZATION

The following tissues were examined by *in situ* hybridization: tongue, fore and hind interdigital cleft, fore and hind coronary band, carpus, eyelid, flank, shoulder, and lung. A biotinylated RNA probe was produced as described previously (6). Briefly, an FMD cDNA fragment 500 nucleotides in length and coding for part of the polymerase enzyme was inserted into the polylinker region of a PGEM-3Z(f) plasmid vector (Promega Corporation, Madison, Wisconsin) at the PstI site. Plasmid was linearized with BamHI endonuclease and negative sense RNA transcripts were generated using SP6 RNA polymerase (Promega Corporation) in the presence of biotin-11-UTP (Enzo Biochem, New York, New York). Unincorporated biotin was removed by passing the probe through Sephadex G50 (Boehringer Mannheim Biochemicals, Indianapolis, Indiana).

Frozen sections were cut and placed on aminotriethoxysilane-treated slides, fixed for 5 min in 4% paraformaldehyde and stored in 70% ethanol at 4°C until used. Prior to hybridization, sections were rehydrated, digested with proteinase K (1 μg/mL in 0.1 M Tris, pH 8.0, 50 mM ethylenediaminetetraacetate [EDTA]) for 15 min at 37°C and then postfixed in 4% paraformaldehyde for 5 min. Slides were then washed in 0.7% acetic anhydride in 0.1 M triethanolamine, pH 8.0. Sections were equilibrated in prehybridization fluid (1 M NaCl, 0.02 mM EDTA, 50% formamide, 10% polyethylene glycol) for a minimum of one hour at 60°C. To denature any double-stranded forms, sections were heated to 100°C for 10 min followed by brief chilling just prior to hybridization. Eight tissue sections per slide were covered with 70 μL of hybridization fluid containing 2 μg of biotinylated probe, 10 μg denatured salmon sperm DNA, 286 μg yeast tRNA and 1.25 mg bovine serum albumin. A siliconized coverslip, sealed around the edges with nail hardener, kept the fluid in place overnight at 60°C. The following day, coverslips were removed, and the slides were washed in decreasing concentrations of standard sodium citrate (150 mM NaCl, 15 mM sodium citrate, pH 7.0) with formamide at 63°C. To detect the biotinylated probe, sections were incubated in a 1:1000 concentration of streptavidin-alkaline phosphatase (Bethesda Research Laboratories, Bethesda, Maryland) for one hour at 37°C followed by addition of chromogenic substrate BCIP/NBT (Bethesda Research Laboratories). Substrate development was stopped by brief immersion in Tris-EDTA buffer (10 mM Tris-HCl and 0.1 mM EDTA, pH 7.5). Slides were rinsed in increasing concentrations of ethanol to decrease unwanted background activity and then coverslipped.

VIRUS ISOLATION

Blood from the animals euthanized at 48, 72 and 96 hpe was collected every 12 h or less, frozen at −70°C and later assayed for virus. Blood was diluted in minimal essential media with 1% antibiotics (penicillin 200 U/mL, streptomycin 200 μg/mL, gentamycin 30 μ/mL) in tenfold dilutions to an end concentration of 10^{-4}. From each dilution, 0.1 mL was inoculated into each of two 25 cm^2 flasks containing first passage secondary embryonic lamb kidney cells. Cultures were examined for cytopathic effects (cpe) at 48 h after inoculation. The lowest dilutions not demonstrating cpe were blind passaged, and the results were observed 72 h after inoculation.

Oesophageal-pharyngeal (O-P) fluids were collected every 24 h with a modified probang cup, placed in 15 mL Tris-buffered tryptose broth and frozen at −70°C until assayed. For virus isolation, 0.2 mL of high antibiotic solution (penicillin 20,000 U/mL, streptomycin 20,000 μg/mL, mycostatin 1,000 μg/mL, polymyxin B 1,000 U/mL, gentamycin 3,000 μg/mL) was added to 1.8 mL of this O-P fluid, which was then homogenized and clarified by centrifugation. A 0.5 mL aliquot of the supernatant was inoculated into each of two 25 cm^2 cell culture flasks of secondary lamb kidney cells. Cultures were examined for cpe 48 h after inoculation.

To confirm the presence of foot-and-mouth disease virus in the aerosol

TABLE I. The results of *in situ* hybridization of ten adult cattle exposed to an aerosol of foot-and-mouth disease virus

Time interval	Hours postexposure to FMD virus								Mock-exposed	
Animal #	6	12	18	24	36	48	72	96	12	24
Tissue	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
RH i-d cleft[a]	+ + +	+ +	+	+	±	+	±	±	0	0
RF i-d cleft[b]	+ + +	0	0	0	0	+ +	+	+	0	0
RH cor band[c]	+ + +	+	+	+ +	+ +	+	+	+	0	0
RF cor band[d]	+ + +	+ +	+ +	+	+	0	0	+	0	0
Tongue	+	+	+	+ +	+	+ +	+ +	+	0	0
Carpus	+	0	0	0	0	+	+	+	0	0
Eyelid	0	+	+	+	+	0	±	±	0	0
Flank	0	0	0	0	0	0	0	0	0	0
Shoulder	NT	0	0	0	0	0	0	0	0	0
Lung	+ +	+ +	+ + +	+ +	+ +	+ +	+ +	+ +	0	0

0 = Negative
± = Equivocal staining; only slight increase over background
\+ = Positive weak staining
\+ + = Positive staining
\+ + + = Extensive positive staining
NT = Not tested

[a] Right hind interdigital cleft
[b] Right fore interdigital cleft
[c] Right hind coronary band
[d] Right fore coronary band

chamber, 20 mL of cell culture fluid (Hank's balanced salt solution with 0.5% lactalbumin hydrolysate and 28 mM-HEPES) were placed in open petri dishes in the chamber during the nebulization. The fluid was stored at −70°C. This material was inoculated onto secondary lamb kidney cells grown in 96-well plates, using 0.025 mL of tenfold dilutions in minimal essential medium to an end concentration of 10^{-10} in 96-well plates. The cultures were incubated for 72 h at 37°C with 5% CO_2.

RESULTS

CLINICAL AND PATHOLOGICAL FINDINGS

Clinical illness was observed only in animals #6 and #8, euthanized at 48 and 96 hpe, respectively. Animal #6 was febrile (41.5°C) with increased salivation just prior to being euthanized at 48 hpe. In animal #8, there was a febrile response (40.5°C) and increased salivation at 72 hpe.

Gross lesions were confined to animals #6, #7 and #8. In animal #6, there were extensive, intact vesicles occupying large portions of both hind interdigital clefts, and on the tongue there was a 2 cm diameter ruptured vesicle just anterior and lateral to the torus. Animal #7 had a turgid 1 cm^2 vesicle on the midline of the hard palate epithelium and a similar-sized, yet ruptured vesicle adjacent laterally. Animal #8 had an extensive, ruptured vesicle in the caudal half of the left hind interdigital cleft and a similar, yet intact, vesicle in the right hind interdigital cleft. The right fore interdigital cleft had a deep vesicle which was visible only upon dissection. In addition, there was a ruptured vesicle in the epithelium overlying the hard palate.

At 12, 18, 24 and 36 hpe the only histological alterations noted were a few foci of intercellular edema in the surface and crypt epithelium of the tonsils beginning at 12 hpe. This was accompanied by coagulation necrosis of the involved epithelial cells from 18 through 96 hpe. Moderate exocytosis of neutrophils in the epithelium and lumens of tonsillar crypts and lymphocytic hyperplasia in the tonsils, adenoids and lymph nodes were found in control and infected animals.

Distinct histological vesicles were seen in other squamous epithelial tissues collected at 48, 72 and 96 hpe. These varied from small foci of coagulation necrosis of keratinocytes, with or without intercellular edema, to ballooning degeneration and bulla formation. For the most part microscopic lesions were associated with sites of grossly apparent vesicles. These sites included the hard palate, and several locations on the tongue and the fore and hind interdigital clefts. Only minute foci of spongiosis adjacent to the stratum basale were found at sites without grossly apparent lesions. These included the coronary bands, carpi, flanks, tail folds, eyelids and teats. While they were not found in the control animals, the subtle and limited nature of these lesions made interpretation of their specificity uncertain.

Sections of lungs from the 12 h control animal and from all of the infected animals had mild peribronchiolar interstitial edema. In the 6, 18, 24, 72 and 96 hpe lung specimens from infected animals the edema was accompanied by meager lymphocyte, plasmacyte and macrophage infiltration. A few neutrophils and eosinophils were also present at 72 and 96 hpe.

IN SITU HYBRIDIZATION

Ten tissues from each of the ten animals were examined by *in situ* hybridization. Results are presented in Table I.

The most extensive and intense staining was present in the foot tissues of animal #1 collected at 6 hpe — both of the interdigital clefts and both of the coronary bands from this animal had strong cytoplasmic staining of cells of the stratum spinosum. The extent of the staining varied from segmental, with large rafts of cells displaying positive signal, to diffuse (Fig. 1). As the infection progressed, there was a tendency for this signal to be less intense and more localized, so that in tissues collected from 24 h on, the signal was often limited to weakly-staining, isolated groups of approximately 5–30 cells of the stratum spinosum. Vesicles were evident in the section of right hind interdigital cleft of calf #8 at 48 hpe and in the right fore interdigital cleft at 96 hpe. There was

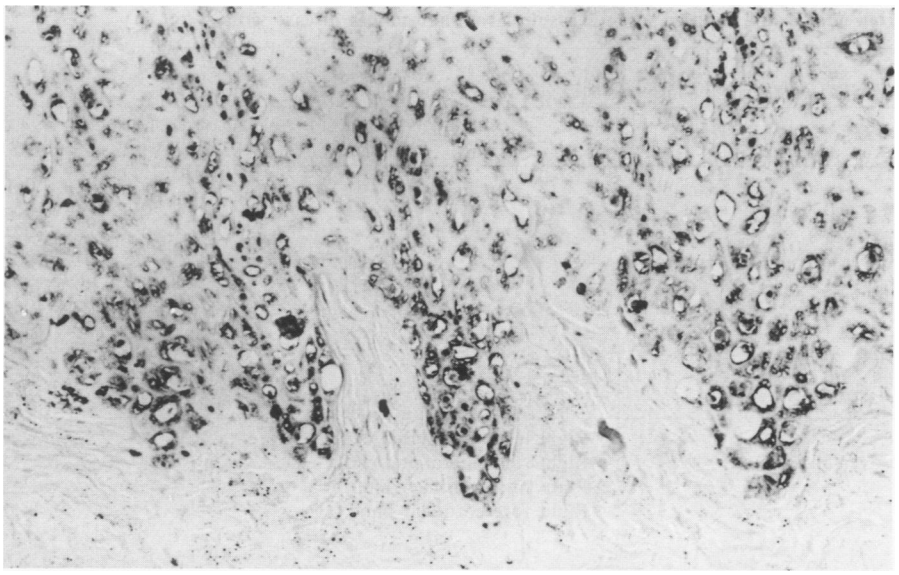

Fig. 1. ***In situ*** **hybridization on right hind interdigital cleft of animal exposed six hours previously (#1) to an aerosol of foot-and-mouth disease virus. No counterstain. ×100.**

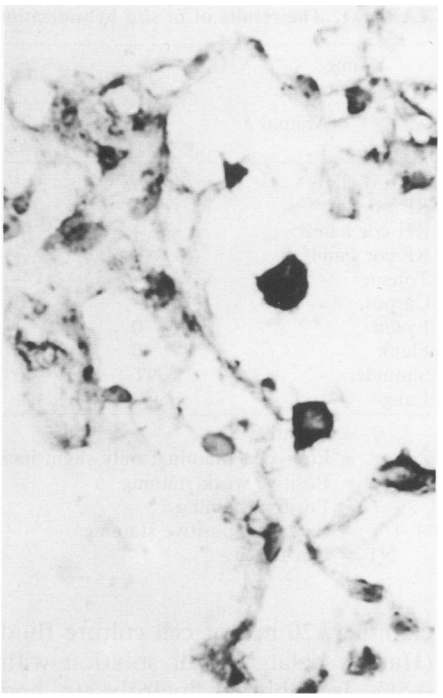

Fig. 2. ***In situ*** **hybridization on lung of animal exposed 96 hours previously (#8) to an aerosol of foot-and-mouth disease virus. No counterstain. ×100.**

weak staining of the cells surrounding the vesicle in these sections.

Staining in the tongue epithelium was definitive yet less dramatic than in interdigital clefts or coronary bands. Positive signals were present in the tongue from all eight of the aerosol-exposed animals. These began as focal aggregates of 10–30 weakly staining cells in the deep stratum spinosum in 6 hpe specimens and progressed to slightly more diffuse, yet still multifocal areas of staining, often a little higher in the stratum spinosum in specimens collected from 12 to 72 hpe. At 96 hpe, signal was limited to weak, multifocal staining of the upper stratum spinosum.

Sections of carpus displayed only sporadic staining through the series. There was slight diffuse positivity in the cells of the stratum spinosum of the animal euthanized 6 hpe. Sections from subsequent animals were negative until 48 and 72 hpe, when there was focal positive staining in the stratum spinosum. A similar distribution at 96 hpe included weak staining around a microscopic vesicle.

In the eyelid, there were small groups of weakly staining cells in the stratum spinosum and stratum granulosum at all sampling intervals except 6 and 48 hpe. Sections of flank and shoulder were consistently negative throughout the series.

The pattern of ISH in the lung was as follows: at 6 hpe, there were multiple foci of strong signal along alveolar septa. The distribution of staining could best be characterized as "patchy". It was not possible to distinguish if the signal was in alveolar epithelial cells, endothelium, or intravascular macrophages. The pattern of staining at 12 and 18 hpe was similar to what was seen at 6 hpe yet increased in intensity. At 36, 48 and 72 hpe there was continued localized staining of alveolar septa, and staining of alveolar macrophages became apparent. At 96 hpe there was still some staining of septal cells, but alveolar macrophages were prominent in their positivity (Fig. 2).

VIRUS ISOLATION

Blood from animals #6, #7 and #8 was tested for virus isolation. Viremias were detected only in #6 and #8. Foot-and-mouth disease virus was not isolated from either of these animals at 6 and 24 hpe. At 30 h and 48 h, #6 had FMDV titers of 10^2 and $10^{3.5}TCID_{50}/mL$, respectively. Animal #8 was negative at 30 h but had titers of 10^1, $10^{1.3}$, $10^{1.5}$, $10^{2.5}$ and $<10^{0.5}TCID_{50}/mL$ at 48, 54, 72, 78 and 96 hpe, respectively. Blood from animal #7 was consistently negative.

Oesophageal-pharyngeal fluids taken daily from animals #6, #7 and #8 were assayed. All were negative prior to exposure and positive for the presence of FMD virus at 24, 48, 72 and 96 hpe.

Foot-and-mouth disease virus was cultured from media from the petri dishes placed in all aerosol chambers to confirm the infectivity of the aerosols. Chamber fluids from the two mock exposures using only cell culture media were negative for FMD virus.

DISCUSSION

Other investigators (1,4) have used the techniques of virus isolation or fluorescent antibody to detect the presence of FMD virus in skin sections at daily intervals subsequent to infection. In these studies, by fluorescent antibody, the viral antigen was detected in lip sections as early as 1 dpi (4). By virus isolation, an examination of numerous skin samples taken from areas not normally associated with lesion development in FMD infection, these skin samples were positive for virus 2 to 5 dpi (1). A major advantage in our study was the ability to apply the very sensitive technique of viral nucleic acid detection to tissues harvested from animals euthanized at early and frequent intervals following experimental aerosol exposure. A pro-

minent finding in this study was the presence of large amounts of viral nucleic acid detected by *in situ* hybridization in multiple epidermal sites only 6 h after aerosol exposure to FMD virus. At this time, all of the interdigital clefts and coronary bands examined had strong, clear signals consisting of granular cytoplasmic staining in focally extensive to diffuse areas of the stratum spinosum. These findings indicated that within six hours of aerosol exposure, FMD virus was replicating at sites of predilection for development of lesions. These ISH signals were present well before the onset of viremia detectable by virus isolation 30 hpe. The ISH signals decreased in intensity and extent with time. In the coronary bands, which did not develop distinctive lesions, either grossly or histologically, staining decreased to focally positive groups of cells in the upper stratum spinosum. In the right hind interdigital cleft, the strong, clear signal present at 6 hpe decreased with time to weaker focal areas of staining, occasionally surrounding a vesicle.

Similarly, ISH signal was present in groups of stratum spinosum cells in the sample of tongue taken only six hours after aerosol exposure. This signal was detected at all time intervals in the aerosol-exposed animals and tended to be present as multifocal groups of cells with cytoplasmic signal. Intensity varied from animal to animal with strongest positivity at 48 hpe. There was some tendency for staining to progress from basal to more apical portions of the stratum spinosum with time.

It is interesting to speculate on how the virus was able to gain access to certain predilection sites within such a few hours after inhalation. In previous studies using guinea pigs and swine, the pattern of staining with ISH for FMD has been predominantly "segmentally diffuse", with groups of 10–100 contiguous cells of the stratum spinosum displaying positivity, whereas adjacent seemingly similar areas would be devoid of signal (7,8). There was a pronounced tendency for this "segmentally diffuse" pattern to be present in the aerosol-exposed cattle as well. In particular, tongue and coronary bands were striking in this regard. It has been suggested that precursors of Langerhans cells might be functioning in viral transport from the blood to the predilection sites. In experiments using infected guinea pig foot pads, it was found by fluorescent antibody technique that FMD virus was initially present only in Langerhans cells in the skin and later was present in both Langerhans cells and epidermal cells (9). The "segmentally diffuse" pattern could be explained as a group of epidermal cells all in contact with and subsequently infected by one infected Langerhans cell.

Some epidermal sites not normally thought of as predilection sites for lesion development in FMD were examined. In the flank and shoulder, no signal was detected. In the carpus, there was focal weak positivity at 6 hpe, 48 hpe and 72 hpe. In the 96 hpe specimen of carpus, there was weak positive staining around a vesicle. In the eyelid, there were weak signals consisting of small groups of cells in the stratum spinosum and stratum granulosum at all time intervals except 6 and 48 hpe. In a previous study various epidermal sites, including carpus, were positive by virus isolation one to five days after intradermolingual or intramuscular inoculation (1). This same study described more widespread histological lesions than were found in the present study. The differences might be explained by the different routes of inoculation and the resulting earlier and higher titer viremias which developed in the previous study. However, strain variations and variability among the limited number of animals sampled in the present study may have also played a role.

The presence of viral nucleic acid in the lung following aerosol exposure was determined by ISH. In previous work Burrows *et al* (5) infected animals by instilling FMD virus in the lung via tracheotomy, and isolated higher titers of virus from pharyngeal tissue than from lung. They concluded that the lung may not be a primary site of initial viral growth. Using a tracheostomy to separate upper from lower respiratory tracts, Sutmoller and McVicar (3) instilled virus directly into lung and subsequently were able to isolate virus from the pharyngeal area. From this, they concluded that FMD virus enters through the lung and that the pharynx is seeded hematogenously. In this study using ISH, staining was multifocal involving cells in the alveolar septa early in infection (i.e. 6–18 hpe), while it was more likely to be found in alveolar macrophages later in infection (i.e. 36–96 hpe). From this pattern, it would appear that the virus initially localized in septal cells and later, coinciding with the onset of detectable viremia, became prominent in alveolar macrophages.

In summary, this study has underscored the very rapid nature of FMD infection, with establishment of significant amounts of viral nucleic acid at predilection sites for lesion formation within 6 h of inhalation of virus. Other epidermal sites not usually thought of as foci for lesion development may also be seeded by virus very early in infection, well before the onset of detectable viremia. With respect to the lung, there is abundant viral nucleic acid, primarily in the septa, early in infection. This viral nucleic acid is predominantly localized to alveolar macrophages later in infection.

REFERENCES

1. **GAILIUNAS P.** Microscopic skin lesions in cattle with foot-and-mouth disease. Arch Ges Virusforsch 1968; 25: 188–200.
2. **ESKILDSEN MK.** Experimental pulmonary infection of cattle with foot-and-mouth disease virus. Nord Vet Med 1969; 21: 86–91.
3. **SUTMOLLER P, McVICAR JW.** Pathogenesis of foot-and-mouth disease: the lung as an additional portal of entry of the virus. J Hyg (Camb) 1976; 77: 235–243.
4. **YILMA T.** Morphogenesis of vesiculation in foot-and-mouth disease. Am J Vet Res 1980; 41: 1537–1542.
5. **BURROWS R, MANN JA, GARLAND AJM, GREIG A, GOODRIDGE D.** The pathogenesis of natural and simulated natural foot-and-mouth disease infection in cattle. J Comp Pathol 1981; 91: 599–609.
6. **MEYER RF, BROWN CC, MOLITOR TW, VAKHARIA VN.** The use of in situ hybridization for the detection of FMD in cell culture. J Vet Diagnost Invest 1989; 1: 329–332.
7. **OLANDER HJ, BROWN CC, MEYER RF.** Pathogenesis of foot-and-mouth disease in swine, using *in situ* nucleic acid hybridization. Proceedings 11th Congress of the International Pig Veterinary Society, Lausanne, Switzerland, July 1–5, 1990.
8. **BROWN CC, OLANDER HJ, MEYER RF.** A preliminary study of the pathogenesis of foot-and-mouth disease using in situ hybridization. Vet Pathol 1991; 28: 216–222.
9. **DiGIROLAMO W, SALAS M, LAGUENS RP.** Role of Langerhans cells in the infection of the guinea-pig epidermis with foot-and-mouth disease virus. Arch Virol 1985; 83: 331–336.